Markers of the Low Dose Radiation Response

Project ID: 69906

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Research Objective

The goal of this work is to develop a technology that will allow direct visualization of DNA double-strand break repair complexes in their original places in the nuclei of irradiated cells.

Ionizing radiation has a unique ability to induce damage simultaneously at multiple sites within a spatially restricted region of DNA. The resulting double-strand DNA breaks (DSBs) present a major threat to the integrity and stability of the genome. Our understanding of the origin and fate of DSBs is based primarily on studies at high radiation doses. Despite recent progress toward the elucidation of molecular mechanisms underlying DSB repair, technologies are still not available to visualize individual DSBs and DSB repair complexes in situ, in irradiated cells.

This project will involve development of a technology to allow direct in situ visualization of DSB repair complexes. These studies will help bridge the gap between biochemical studies of repair enzymes and an understanding of the process of repair as it actually occurs within the radiation-injured cell. Specific aims include:

1. The development of tools for in situ visualization of DSB repair complexes. These tools will include fluorescently tagged repair proteins that can be used to visualize repair complex assembly in living cells. They will also include recombinant single-chain antibodies that will be used to detect changes in the conformation and phosphorylation state of DSB repair proteins that accompany the assembly of the repair complex.

2. The use of these tools in combination with fluorescence microscopy to detect and characterize visible subnuclear structures associated with DSB repair. Control and reconstruction experiments will be performed to correlate visible structures with biochemically-defined repair complexes. Strategies will be developed to prolong the lifetime of repair complexes to make them more easily visible. Immunoprecipitation experiments will be performed to identify additional proteins that contribute to formation of repair structures.

3. A demonstration of the practical utility of the tools and assays developed in Aims 1 and 2 by quantitative studies of DSB repair in different cell types. The formation of repair complexes at high and low radiation doses will be compared. The effect of a low radiation dose on the response to subsequent, higher doses will be determined. The relative prevalence of the Ku-dependent end joining and Rad51-dependent recombinational pathways will be investigated.

The ability to visualize single DSB repair complexes formed at the sites of DNA damage will provide an improvement of two orders of magnitude in sensitivity over existing physical methods of measuring double-strand breaks. It will open up exciting prospects for direct investigation of the low-dose radiation response.